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Peer Review File

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**Reviewer A**

Thank you for submitting your manuscript to Journal of Gastrointestinal Oncology. In recent years, with the increasing incidence of colorectal cancer diagnosed in individuals under the age of 50, this study highlighting the characteristics of young colorectal cancer patients emphasizes the need for screening microsatellite instability in this population. However, some of issues should be addressed:  
Comment 1: In the inclusion criteria, “colonoscopy” instead of gastroscopy and “colectomy” instead of gastrectomy would be appropriate; your study includes patients with colorectal cancer, doesn't it?" (page 4, line 92, 95)

Reply 1: Thank you for pointing out that it has been revised in the article.

Changes in the text: page4 line123

Comment 2) It would be more appropriate to mention the sentence “The staging was based on the 8th edition of the TNM classification system of the Union for International Cancer Control (UICC)/AJCC.” in the methods section.

Reply 2: Thank you for your reasonable suggestion, which I have modified in the text.

Changes in the text: page5 line134

Comment 3) The method section should specify how microsatellite instability was assessed (immunohistochemistry, PCR, etc.). Was EMAST-type instability evaluated?

Reply 3: We used fluorescence in situ hybridization to determine MSI in colorectal cancer patients. We did not perform testing for EMAST-type instability.

Changes in the text: page5 line139

Comment 4) Was genetic screening performed, especially in young patients and MSI-H tumors, for Lynch syndrome? How many of your MSI-H cancer patients are associated with Lynch syndrome?

Reply 4: Thank you for your comments. We did not perform an analysis of patients with Lynch syndrome in all MSI-H patients because 1. Patients did not have enough first-degree relatives with cancer for the analysis of the Lynch syndrome. 2. There is also insufficient information on this aspect when collecting patient information.

Comment 5) In the methods section, you had indicated that patients who underwent neoadjuvant treatment or radiotherapy were not included in the study. Did all your patients with locally advanced rectal cancer receive only adjuvant treatment?

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Reply 5: This is a writing error; it should be correct that our patients were those receiving perioperative adjuvant therapy.

Changes in the text: page5 line124

Comment 6) For the young and elderly patient groups included in the study for survival analysis, what adjuvant treatments were administered and for how long? What percentage of patients were able to complete their adjuvant treatments? Did patients diagnosed with rectal cancer receive adjuvant radiotherapy? Were there any differences between the elderly and young patient groups in terms of drug choices and treatment completion durations for adjuvant therapies? These are factors expected to impact survival and should be explicitly included in survival analyses.

Reply 6: Our patients were those who received perioperative adjuvant therapy, so by default we ruled out the effect of this factor on survival. This will be a factor in our analysis in the following study.

Comment 7) It is appropriate to write the abbreviations of the the tables in alphabetical order.

Reply 7: Thank you for your suggestion, we have made corrections to this place.

Changes in the text: page13 line385; page14 line393; page15 line403

Comment 8) When survival data is provided under the title 'Univariate Analysis,' the p-value should be given after specifying survival times and 95% CI in parentheses.

Reply 8: Thank you for pointing it out. It has been added in that aspect within the text.

Changes in the text: page6 line190

Comment 9) The anatomical localization referred to as 'proximal' and 'distal' colon in the 'Tumor Location' section of the tables should be explained in the methods section.

Reply 9: The tumors were divided into proximal and distal segments based on the splenic flexure of the colon. And in the Methods section of the text.

Changes in the text: page5 line137

Comment 10) A survival table for all patients can be omitted because poor prognostic factors are known in colorectal cancer.

Reply 10: Although the poor prognostic factors of colorectal cancer are known, we believe that this table needs to be reflected, so we have included them in this article.

Comment 11) In tables, 'sex' is more accurate than 'gender'; 'Gender' generally refers to social gender, while 'sex' specifies biological gender.

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Reply 11: Your suggestion is reasonable and I have modified it in the text.

Changes in the text: page13 line384; page13 line392; page14 line402

Comment 12) The results mentioned in the results section and presented in the tables should not be reiterated in the discussion section unless a specific discussion paragraph related to that result is to be written.

Reply 12: We have made the corresponding changes to the relevant part of the article.

Changes in the text: page8 line234

### **Reviewer B**

- 1) Comment 1: First, the title needs to indicate the comparisons between the two groups and the clinical research design of this study, i.e., a retrospective comparative cohort study.

Reply 1: Thank you for your reasonable suggestion, which I have modified in the text.

Changes in the text: page1 line4

- 2) Comment 2: Second, the abstract needs some revisions. The authors need to explain the clinical needs for comparing old and young patients and what the current knowledge gap is. The methods need to describe the inclusion of subjects, follow up procedures, data collection of clinical variables and prognosis outcomes, and test method for MSI-H. The results need to briefly describe the clinical characteristics and prognosis of the two groups. HR values for the identified prognostic factors should also be provided. The conclusion needs more detailed comments for the clinical implications of the findings.

Reply 2: Thank you for your feedback. We have made the necessary changes.

Changes in the text: page2 line46

- 3) Comment 3: Third, the introduction needs to analyze why it is clinically important to compare the young and old groups and what the current knowledge gap is on this research focus.

Reply 3: Thank you for your comments, which have been added in the introduction.

Changes in the text: page2 line38

- 4) Comment 4: Fourth, in the methodology, the authors need to describe the clinical research design, sample size estimation, details of follow up, and how the

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prognosis outcomes were assessed. The cut-off ages for young and old groups in this study are unusual, the authors need to explain why and why they excluded patients aged between 41-68 years. In statistics, the authors need to describe the test of the baseline comparability of the two groups, and multiple regression analysis for adjusting the baseline confounding factors. The authors need to provide details of the Cox regression and ensure  $P < 0.05$  is two-sided.

Reply 4: The situation is that our finding was found in clinical observation, but we found that there was no clinical significance when comparing middle-aged and young patients during statistical analysis. We considered that the proportion of MSI-H patients in middle-aged patients may be too low to be meaningful, so we analyzed the elderly patients with a higher proportion.

- 5) Comment 5: Finally, please cite several related papers: 1. Fu Y, Ye Y, Liu X, Zhu G, Xu Y, Sun J, Wu H, Feng F, Wen Z, Jiang S, Li Y, Zhang Q. Analyzing microsatellite instability and gene mutation in circulating cell-free DNA to monitor colorectal cancer progression. *Transl Cancer Res* 2021;10(6):2812-2821. doi: 10.21037/tcr-20-2762. 2. Duan Y, Xu D. Microsatellite instability and immunotherapy in gastric cancer: a narrative review. *Precis Cancer Med* 2023;6:14. 3. Zhou Z, Li K, Wei Q, Chen L, Shuai Y, Wang Y, He K, Si L, Zhong Y, Lu J. Tumor mutation burden determined by a 645-cancer gene panel and compared with microsatellite instability and mismatch repair genes in colorectal cancer. *J Gastrointest Oncol* 2021;12(6):2775-2787. doi: 10.21037/jgo-21-572.

Reply 5: Thank you for recommending the article. The content is excellent and has complemented our own research. I have already included citations to these articles in our work.

Changes in the text: page10 line265; page12 line369